Genetic Testing for Oculocutaneous Albinism Type 1 and 2 and Hermansky–Pudlak Syndrome Type 1 and 3 Mutations in Puerto Rico

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Hermansky–Pudlak syndrome (HPS) (MIM #203300) is a heterogeneous group of autosomal recessive disorders characterized by oculocutaneous albinism (OCA), bleeding tendency, and lysosomal dysfunction. HPS is very common in Puerto Rico (PR), particularly in the northwest part of the island, with a frequency of ~1:1,800. Two HPS genes and mutations have been identified in PR, a 16-base pair (bp) duplication in *HPS1* and a 3,904-bp deletion in *HPS3*. In Puerto Ricans with more typical OCA, the most common mutation of the tyrosinase (*TYR*) (human tyrosinase (*OCA1*) gene) gene was G47D. We describe screening 229 Puerto Rican OCA patients for these mutations, and for mutations in the *OCA2* gene. We found the *HPS1* mutation in 42.8% of cases, the *HPS3* deletion in 17%, the *TYR* G47D mutation in 3.0%, and a 2.4-kb deletion of the *OCA2* gene in 1.3%. Among Puerto Rican newborns, the frequency of the *HPS1* mutation is highest in northwest PR (1:21; 4.8%) and lower in central PR (1:64; 1.6%). The *HPS3* gene deletion is most frequent in central PR (1:32; 3.1%). Our findings provide insights into the genetics of albinism and HPS in PR, and provide the basis for genetic screening for these disorders in this minority population.

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INTRODUCTION

Hermansky–Pudlak syndrome (HPS) (MIM #203300) is a genetically heterogeneous group of autosomal recessive disorders characterized principally by oculocutaneous albinism (OCA), bleeding tendency, and progressive pulmonary fibrosis (Hermansky and Pudlak, 1959). HPS is a rare genetic disease worldwide, but it is the most common single-gene disorder on the island of Puerto Rico (PR), particularly in the northwest region, where it occurs with a frequency of 1:1,800 (Witkop *et al.*, 1990a, b). HPS thus represents a significant public health problem in PR (Witkop *et al.*, 1990a; Spritz, 1998). Other forms of OCA also occur in PR, but are much less common (Witkop *et al.*, 1990a).

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Abbreviations: bp, base pair; BT, bleeding time; HPS, Hermansky–Pudlak syndrome; OCA, oculocutaneous albinism; P, *human pink-eyed dilution* (OCA2) *gene; PR, Puerto Rico;* TYR, *human tyrosinase* (OCA1) *gene*

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The consequences of reduced skin pigmentation in OCA and some forms of HPS, particularly photosensitivity and social stigmatization due the noticeable skin color differences, are relatively significant in PR, where the sun exposure rate is high and where relatively dark skin pigmentation is the norm. Ocular consequences of OCA are typical in PR, including low vision, nystagmus, strabismus, and photophobia (Izquierdo *et al.*, 1995), again exacerbated by the high level of ambient sunlight. The systemic complications of HPS are a particular problem; average patient survival is 30–50 years, death usually resulting from restrictive lung disease (68%), hemorrhage (17%), or granulomatous colitis (15%) (Witkop *et al.*, 1990a). There is no specific or effective treatment for most of the manifestations of HPS, although pirfenidone may slow the progression of pulmonary fibrosis (Gahl *et al.*, 2002).

Studies of OCA and HPS patients from around the world have demonstrated considerable genetic heterogeneity, with four genes known to cause OCA and seven known to cause HPS in humans (Spritz *et al.*, 2003; Bonifacino, 2004). Early premolecular epidemiologic studies in PR (Witkop *et al.*, 1990a, b) estimated that, among 349 patients with albinism, there were five types of OCA and two types of ocular albinism: 12% were considered to have OCA1, 4.9% OCA2, 0.6% OCA3, and 80–83% HPS, mostly from northwest PR. HPS in PR was subsequently shown to result from founder effects for single mutations of two genes, *HPS1* and *HPS3*,

which facilitated localization and identification of these genes by linkage disequilibrium mapping and positional cloning (Fukai *et al.*, 1995; Wildenberg *et al.*, 1995; Anikster *et al.*, 2001). Puerto Rican HPS1 patients have all been homozygous for a 16-bp duplication/frameshift in the *HPS1* gene (Oh *et al.*, 1996) and Puerto Rican HPS3 patients have all been homozygous for a 3,904-bp deletion in the *HPS3* gene (Anikster *et al.*, 2001). Similarly, a single mutation of the tyrosinase (*TYR,* human tyrosinase (*OCA1*) gene) gene, G47D, was found to be prevalent among northwest Puerto Rican patients with OCA1 (Oetting *et al.*, 1993). This mutation has been found in many populations around the world, and may have been propagated by Spanish sailors.

We have studied the clinical manifestations and molecular genetics of OCA1 and 2 and HPS type 1 and 3 in San Juan, PR. Our objectives were as follows: to determine the prevalence of HPS1 and 3 among patients with OCA referred to the outpatient clinics of the University of Puerto Rico School of Medicine; to determine the presence of the *HPS1* gene 16-bp duplication, the *HPS3* gene 3,904-bp deletion, and the presence of *TYR* and human pink-eyed dilution (*OCA2*) gene (*P*) mutations among Puerto Ricans with OCA; and to determine the carrier frequencies of the two common *HPS1* and *HPS3* mutations among Puerto Rican newborns.

RESULTS AND DISCUSSION

We studied a total of 229 (114 male and 115 female patients) unrelated Puerto Rican patients presenting with albinism. Patients' ages ranged from 1 month to 58 years (mean 8.24 ± 10.34 years). We studied a total 585 individuals in the 229 families. In families with multiple affected members, only one affected individual was included in the study group. All cases appeared consistent with autosomal recessive inheritance, although two families exhibited apparent pseudo-dominance due to an affected parent. Indeed, 80% of families reported albinism in the extended family, such as probands' cousins, aunts, uncles, or grandparents, suggesting that the frequency of disease alleles is relatively high on the island.

We observed considerable phenotypic heterogeneity among the study patients. All patients had low vision with reduced visual acuity, nystagmus, and photophobia. Apparent skin pigmentation was greatly reduced or absent among patients subsequently found to have OCA1 or OCA2. In contrast, skin hypopigmentation was quite variable and often subtle among patients subsequently found to have HPS3, although patients were invariably less pigmented than their parents or unaffected sibs. Three family members of HPS3 patients were found to have undiagnosed HPS3 when genetic testing was carried out and homozygosity for the *HPS3* gene 3,904-bp deletion was established. These three cases had nystagmus, very mild hypopigmentation, and ocular albinism as well as easy bruising.

As most of the HPS patients studied here were children, the severe clinical manifestations of HPS were not found as often as reported in older patients. The most common respiratory symptoms among the pediatric HPS patients included rhinitis, cough, and bronchial wheezing; some patients had been diagnosed with bronchial asthma. Four patients with HPS3 had colitis or gastrointestinal bleeding of unknown etiology at very early ages, including one patient at birth and one prior to 2 years of age. Two out of these four patients who presented lower gastrointestinal bleeding and/or colitis were considered to have mild bleeding associated to the bleeding diathesis. Their signs and symptoms disappeared promptly; thus, they were not evaluated further. However, in the other two patients, the signs and symptoms were significant enough to get gastrointestinal bleeding consultation and biopsy. In these two cases (ages 5 and 20 years, respectively), the gastrointestinal bleeding biopsy was reported by an experienced pathologist as colitis. These patients are being followed by pediatric and adult gastroenterologists.

Approximately half of the 229 patients with OCA included in this report were cases referred directly to the genetics laboratory in our institution by ophthalmologists who asked for HPS genetic testing. Most of these patients were not available for evaluation in our hematology service. Out of the 229 albino patients reported in our article, 109 (47%), most of them with a variable bleeding diathesis, were tested for bleeding time (BT) and/or platelet aggregation tests. Forty-nine of these 109 (45%) were homozygous for the *HPS1* gene 16-bp duplication and 16 (32.7%) for the *HPS3* gene 3,904-bp deletion. Thirty-nine out of these 49 (79.6%) HPS type 1 patients had an abnormal BT, and 13 of the 16 patients with HPS3 (81.2%) had an abnormal result.

Platelet aggregation tests were performed in 33 of the 49 HPS type 1 cases, out of which 26 (79%) had an abnormal aggregation test with two or more platelet aggregation agents. Similarly, among the 16 HPS3 patients, 13 (77%) showed abnormal aggregation with two or more platelet agonists.

These hematological tests were also performed in a group of 16 albino patients who did not have homozygous *HPS1* or *HPS3* gene mutations, including five OCA1 and two OCA2 patients, and nine cases who were heterozygous for the *HPS1* or *HPS3* gene mutations frequent among Puerto Rican patients. The BT was normal in all five homozygous OCA1 and two OCA2 patients tested. Similarly, the platelet aggregation tests were normal in two out of three OCA1 and in both OCA2 patients tested. Corresponding tests performed in the nine patients who were heterozygous for the *HPS1* gene 16-bp duplication showed that six had abnormal BTs, and two out of four (who reported mild to moderate bleeding diathesis) also had an abnormal platelet aggregation.

We tested patient DNA samples for the most common Puerto Rican HPS mutations and, in some cases, screened for additional mutations in the *HPS1*, *TYR*, and *OCA2* genes. Patients who were heterozygous for the *HPS1* gene 16-bp duplication or the HPS3 3,904-bp deletion were screened for mutations in the *HPS1*, *OCA1*, and *OCA2* genes by exon screening. Those who were heterozygous for the *HPS1* gene 16-bp duplication were also screened for the *HPS3* gene 3,904-bp deletion. No exon screening of the *HPS3* gene was performed, since we had only two cases of patients heterozygous for the *HPS3* gene 3,904-bp deletion. Among the 229 unrelated albinism probands, 98 (42.8%) were

homozygous for the common Puerto Rican *HPS1* gene 16-bp duplication (Oh *et al.*, 1996) (Table 1). Another 11 patients (4.8%) were heterozygous for this mutation (Table 1); exon screening and sequencing detected no other pathologic mutations of the *HPS1* gene in these patients. *In toto*, 47.5% of patients were either homozygous or heterozygous for the common *HPS1* duplication. HPS type 1 thus comprised the major apparent OCA disorder in this study, principally (>90%) involving patients derived from northwest PR.

An additional 39 (17%) patients were homozygous for the common *HPS3* gene deletion (Anikster *et al.*, 2001); two (0.9%) were heterozygous for this mutation. Nineteen (~49%) of these cases were from central PR and the rest were from various townships, including Ponce, Salinas, Patillas, Cayey, Cidra, Bayamón, Caguas, San Lorenzo, Toa Alta, and San Juan. These findings indicate that this mutation probably arose in central PR, but is now distributed widely across the island.

Analysis of the principal OCA genes (*TYR* and *P/OCA2*) by exon screening and/or mutation-specific multiplex PCR detected seven patients (3.0%) homozygous for the *TYR* G47D mutation. This mutation, originally observed in Caucasian patients, was subsequently observed in 11 Puerto Rican OCA1 patients (Oetting *et al.*, 1993) and was attributed to migration to PR from the Canary Islands. However, the G47D mutation is also extremely common among Moroccan Jews (Gershoni-Baruch *et al.*, 1994), and it may alternatively be that this mutation was introduced to PR from the Canary Islands, and to North Africa directly from Spain or via some other route. Whatever its origin, the G47D mutation appears to be the most common *TYR* gene mutation among Puerto Ricans with OCA1.

Three patients (1.3%) were found to be homozygous, and 14 (6.1%) heterozygous, for the *OCA2* 2.7-kb deletion commonly found in African (Spritz *et al.*, 1995; Stevens *et al.*, 1997) and African-American (Lee *et al.*, 1994; Durham-Pierre *et al.*, 1996) patients with OCA. This is the first report of the 2.7-kb *P/OCA2* gene deletion in PR, although the finding of this mutation in PR is not surprising given the historical admixture of Africans into the Puerto Rican population (the slave trade in PR began in the early 16th century (Izquierdo *et al.*, 1993), principally from central west Africa and Bantu-speaking Africa (Nagel and Ranney, 1990)). The *OCA2/P* gene deletion is remarkably frequent among the Bantu-speaking peoples of Tanzania (Spritz *et al.*, 1995) and South Africa (Stevens *et al.*, 1995).

Table 1. Results of mutation analysis of the *HPS1*, *HPS3*, *TYR*, and *OCA2/P* genes in 229 Puerto Ricans with albinism

Genotype	Number	Percentage of total
Homozygous *HPS1* 16-bp duplication	98	42.8
Heterozygous *HPS1* 16-bp duplication	11	4.8
Homozygous *HPS3* 3,904-bp deletion	39	17.0
Heterozygous *HPS3* gene 3,904-bp deletion	2	0.9
Homozygous *TYR* G47D mutation	7	3.0
Homozygous *OCA2/P* gene 2.7-kb deletion	3	1.3
Heterozygous *OCA2/P* gene 2.7-kb deletion	14	6.1
Unidentified mutation	55	24.0
Total	229	100

To determine the frequency of the common *HPS1* and *HPS3* mutant alleles in PR, we tested 2000 dried blood samples of newborns from the indigenous island population (indigenous meaning children born from Puerto Rican parents living in the island of PR and not in the mainland United States). As shown in Table 2, the frequency of the common *HPS1* gene 16-bp duplication allele is 0.0476 (1/21), essentially as estimated by Witkop *et al.* (1990a, b) from epidemiologic surveys of patients from northwest PR (Table 2). The frequency of the *HPS1* mutant allele is lower among individuals from east and southeast PR. As expected, the frequency of the *HPS3* deletion allele is higher in the central region of PR (1/32) than elsewhere on the island, and is least prevalent (1/187) in northwest PR (Table 2). Interestingly, the overall summary frequencies of the *HPS1* and *HPS3* mutant alleles for the island *in toto* are essentially identical – 1/59. The *HPS1* 16-bp duplication allele appears to be in Hardy–Weinberg equilibrium in the populations of northwest and central PR ($P<0.05$), but not in the total population and that of the main region of the island (Supplementary material). We do not know the reason for the discrepancies between the observed and expected frequencies, which could be due to one or more of the Hardy–Weinberg conditions not having been met.

The genotype frequencies for the *HPS1* gene mutation are significantly different in the three principal geographic regions of the island ($\chi^2=28.8$; $P=0.000$; see Supplementary material). These differences may be due to the founder effect already demonstrated to be present in northwest PR for HPS, which results in a high genotype frequency and an expected difference in the genotype frequency in other regions of the island. Alternatively, these differences may be due to recent major changes in the population structure of the main region and, thus, in the island as a whole. In contrast, the *HPS3* deletion allele appears to be in Hardy–Weinberg equilibrium in the three geographic regions of the island ($\chi^2<0.2$, which is $\ll 3.84$, for $P<0.05$; see Supplementary material), which suggests that conditions for Hardy–Weinberg equilibrium were met.

Our findings provide insights into the genetic epidemiology of albinism in PR. In the island of PR, HPS1 is the most frequent cause of albinism, accounting for at least 43% of cases, particularly among patients from the northwest part of the island. HPS3 is next in prevalence, accounting for 17% of the total, particularly among patients from central PR. Island-wide, however, the carrier frequencies of the common *HPS1* and *HPS3* gene mutations were essentially equivalent (1/59). The scientific literature contains many reports of the high prevalence of HPS in northwest PR (Witkop *et al.*, 1990a, b;

Table 2. Results of screening Puerto Rican newborns for the *HPS1* gene 16-bp duplication and the *HPS3* gene 3,904-bp deletion

	HPS1 16-bp duplication genotype							
Region of the island	**NN**	**NM**	**MM**	**Total**	***P***	***q***	**Estimated population frequency**	**Carrier frequency**
Northwest	379	19	0	398	0.9761	0.0239	1:1,751	4.8% (1:21)
Central	63	1	0	64	0.9922	0.0078	1:16,393	1.6% (1:64)
Main	1,533	14	1	1,548	0.9948	0.0052	1:37,223	0.9% (1:110)
Total	1,975	34	1	2,010	0.9910	0.0090	1:12,346	1.7% (1:59)
	HPS3 3,904-bp deletion genotype							
Region of the island	**NN**	**NM**	**MM**	**Total**	***P***	***q***	**Estimated population frequency**	**Carrier frequency**
Northwest	372	2	0	374	0.9973	0.0026	1:149,354	0.53% (1:189)
Central	62	2	0	64	0.9843	0.0156	1:4,110	3.1% (1:32)
Main	1,538	30	0	1,568	0.9904	0.0095	1:11,087	1.91% (1:52)
Total	1,972	34	0	2,006	0.9915	0.0084	1:14,211	1.7% (1:59)

NN=homozygous normal; NM=heterozygous for the mutation; MM=homozygous for the mutation.

Oh *et al.*, 1996; Anikster *et al.*, 2001 and many others); hence, the increased awareness of the high incidence of HPS among Puerto Ricans in the island's medical community has increased the reporting of HPS in that region of the island, where ~17% of the island population resides. In contrast, awareness of the existence of a second type of HPS in central PR is low, and was not reported in the scientific literature until 2001. The carrier frequency of the *HPS1* gene 16-bp duplication among newborns was higher in northwest PR (1:21) than the carrier frequency of the *HPS3* gene 3,904-bp deletion in central PR (1:32), which can cause a higher number of cases in this more densely populated region of the island, in combination with occult or known inbreeding, which has been reported in both types of HPS in Puerto Rican patients. These facts, combined with the milder phenotype of HPS type 3 in Puerto Rican patients, has contributed to HPS cases not being properly diagnosed early in childhood, as evidenced by the undiagnosed cases mentioned above and may account for the difference in the number of HPS type 1 *versus* HPS type 3 cases referred to the genetics laboratory reported here.

At least 3% of Puerto Rican albinism patients have OCA1, principally involving the G47D substitution. At least 1.3% of patients have OCA2, principally involving the 2.7-kb gene deletion. Altogether, we were able to establish molecular diagnoses for ~64% of the albinism patients in our Puerto Rican cohort. Another 12% had heterozygous mutations in the HPS1, *HPS3*, or *OCA2/P* genes, but no second mutation was found. These patients either had cryptic mutations that were not detected by the PCR-based exon screening methods used or are chance carriers and have mutations in other genes not studied here. A total of 24% of patients had no detectable mutations in any of the genes tested, and again either have mutations not detected by the methods used here or have mutations in other genes not studied.

Our data show that the *HPS1* 16-bp duplication mutation is carried by 1/21 newborns in northwest PR, similar to the previous estimate of 1/22 based on clinical survey (Witkop *et al.*, 1990b) and 1/59 on the island overall. Based on 2000 US Census data, we thus estimate that there may be 308–464 cases of HPS1 on the island. We also provide the first estimate of the carrier frequency of the *HPS3* gene deletion in central PR – 1/32. Surprisingly, the carrier frequency of the *HPS3* deletion allele is also high in the main region – 1/52. The majority of HPS3 cases may thus derive from areas of PR other than the northwest or central regions. The results of our mutation testing among newborns can be extrapolated to the number of children born in the island each year (~50,000/year). Assuming Hardy–Weinberg equilibrium, we estimate that ~7–8 children with HPS (HPS1 and HPS3) will be born on the island each year.

Finally, because >90% of the patients in our study were <18 years of age, some of the severe long-term complications of HPS, such as pulmonary fibrosis and granulomatous colitis, were uncommon in our study population. In several instances, patients' relatives were identified as having OCA only after the proband had been investigated due to clinical hypopigmentation, bruising, low vision, or nystagmus. Thus, some cases, particularly of the clinically milder HPS3, may not be recognized until relatively late, even in adulthood. We

strongly recommend that all Puerto Ricans presenting with even mild hypopigmentation, reduced visual acuity and/or nystagmus, and easy bruising undergo molecular testing for the common *HPS1* and *HPS3* gene mutations. These evaluations will permit correct diagnosis, appropriate management, and genetic counseling of patients from this specific Hispanic population at high risk for these life-threatening disorders.

MATERIALS AND METHODS

Human subjects

This study group included children 0–18 years of age referred to the Coagulation Clinic of the University of PR Pediatric Hospital in San Juan, PR between 1997 and 2004 with either ocular albinism or OCA. Some patients with ocular albinism or OCA were referred by physicians at the Ophthalmology Clinics of the PR Medical Center, Ponce School of Medicine, and the National Organization for Albinism and Hypopigmentation-PR Chapter. All subjects were Puerto Rican, and included both genders. Hematologic and coagulation tests included complete blood count, prothrombin time, activated partial thromboplastin time, BT, platelet aggregation tests, and Factor VIII and von Willebrand factor activities. This study was conducted according to the Declaration of Helsinki Guidelines for the use of human subjects and was approved by the University of PR Medical Sciences Campus Institutional Review Board. Participants gave their written informed consent or assent.

DNA analyses

Genomic DNA was isolated from blood using QIAamp Blood Kits (Qiagen, Valencia, CA). Purified DNAs were electrophoresed in 1% agarose to assess DNA yield and quality, and DNA concentrations were assessed by fluorometry using Hoechst 33258 dye and a Hoeffer DynaQuant fluorometer (Cesarone *et al.*, 1979). Samples were stored at −70°C until used. Exon 15 of the *HPS1* gene was amplified by PCR as described (Oh *et al.*, 1996), and PCR products were analyzed in 3.5% agarose gels (2% LM agarose, 1.5% agarose) to separate the products of the normal (269 bp) and duplication mutant (285 bp) alleles. The *HPS3* gene 3,904-bp deletion was assayed as described (Anikster *et al.*, 2001) using 1.6% agarose gels. Patients heterozygous for the common *HPS1* mutation were further studied by amplification of all exons and DNA sequence analysis as described (Bailin *et al.*, 1997).

We screened all *HPS1* duplication-negative/*HPS3* deletion-negative patients for mutations in the *HPS1*, *TYR* (*OCA1*), and *OCA2*/*P* genes by amplifying and directly sequencing exonic PCR products (Oetting *et al.*, 1991; Gershoni-Baruch *et al.*, 1994; Lee *et al.*, 1995a, b). The common 2.7-kb *OCA2*/*P* gene deletion was assayed as described (Durham-Pierre *et al.*, 1996), and was confirmed by direct automated DNA sequencing of the 420-bp PCR product derived from the deletion allele using the Thermo-Sequenase II sequencing kit (Amersham Biosciences, Piscataway, NJ).

Analysis of dried blood samples of newborns

Blood filters were obtained from newborns from the eight health regions of the island of PR, using a randomized sampling method based on the population distribution in the island reflected in the 2000 US Population Census and in the number of births registered for each of the 46 PR hospitals with childbirth facilities. DNA was extracted from ~2,000 dried blood samples of newborns on Guthrie cards using Chelex resin (Dracopoli *et al.*, 1998) and stored for less than 1 month. PCR analyses were carried out as described (Oh *et al.*, 1996; Anikster *et al.*, 2001), using 20 μl Chelex-extracted DNA.

To randomize the sampling, we collected samples sequentially from the dried blood samples of the PR Newborn Screening Program until the desired number was obtained, excluding samples that came from individuals of ethnicities other than Puerto Rican. Three geographic regions were defined for the purposes of this study, following the regionalization of HPS1 and HPS3 in PR proposed by Anikster *et al.* (2001). The northwest region includes 17 towns (population 701,945, 18.43%), the central region includes four towns (population 105,113, 2.8%), and the main region includes 57 towns (population 3,001,552, 78.8%) (see Supplementary Material for details). The carrier frequency of HPS in northwest PR had been estimated at 1:21 (Witkop *et al.*, 1990a, b), which we used to estimate the proportion of individuals carrying the *HPS1* gene mutation. The average number of live births per year in the island of PR from 2001 to 2003 was 50,724 (PJ Santiago-Borrero, personal communication). A sample of 2000 newborns thus represents 3.9% of the average number of newborns in 2001–2003. We assumed that the frequency of carriers in PR could not be higher than 0.05, for a 95% confidence interval with $d = 0.01$. We obtained a sample size of $n = 1{,}786$, using the large sample normal approximation adjusted for a finite newborn population size (N) of 50,000. Data were analyzed using the Hardy–Weinberg equation and contingency tables constructed using standard methods.

CONFLICT OF INTEREST

The author states no conflict of interest.

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SUPPLEMENTARY MATERIAL

Table S1. Names of Commonwealth of Puerto Rico towns included in the geographic regions defined for the purposes of this study.

Table S2. Resuts of screening 2,010 Puerto Rican newborns for the *HPS1* 16-bp duplication.

Table S3. Results of screening 2,006 newborns for the *HPS3* gene 3,904-bp deletion.

Figure S1. Map of the US Commonwealth of Puerto Rico. The map shows the location of the selected towns in the Northwest in gray, those in the Central region in black and those in the Main region in white.

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